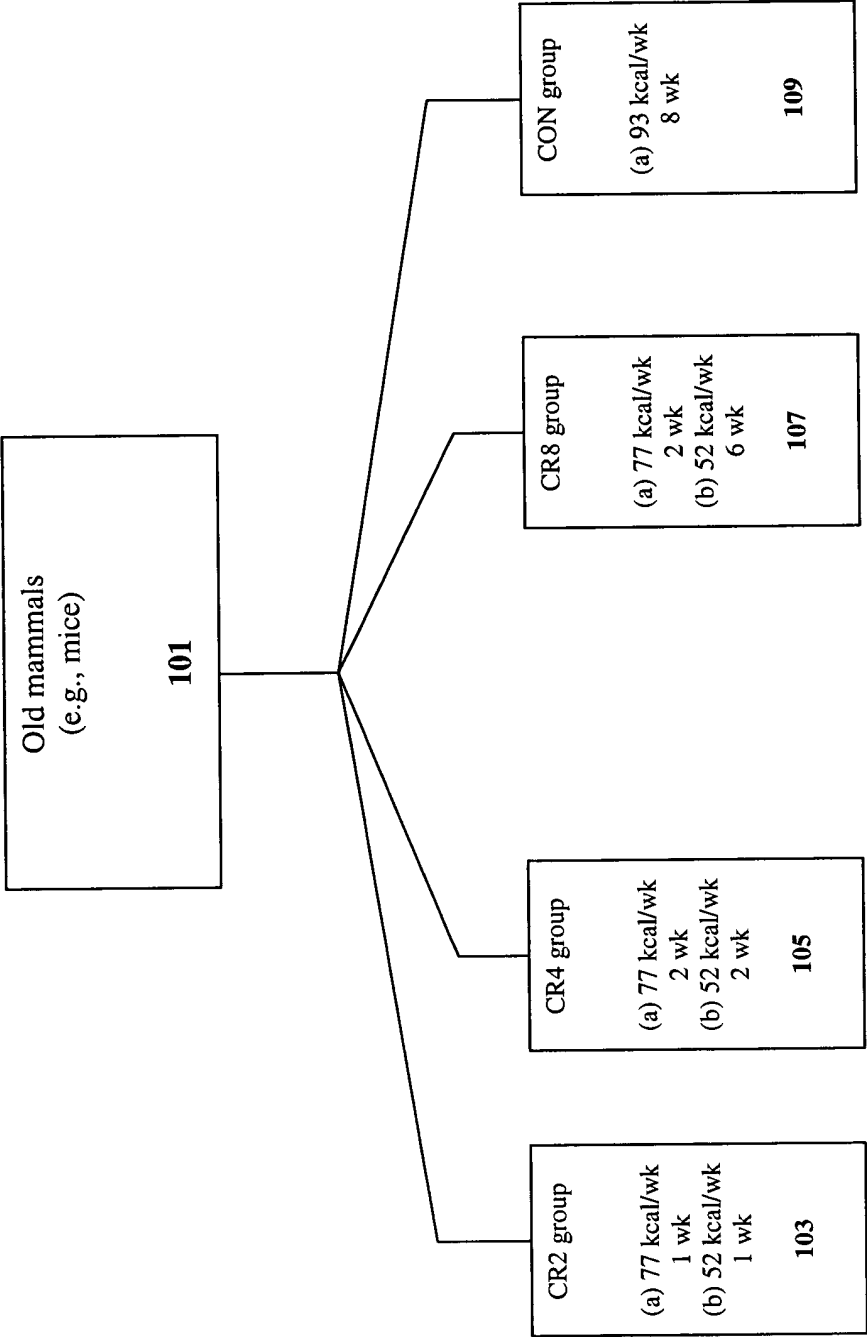
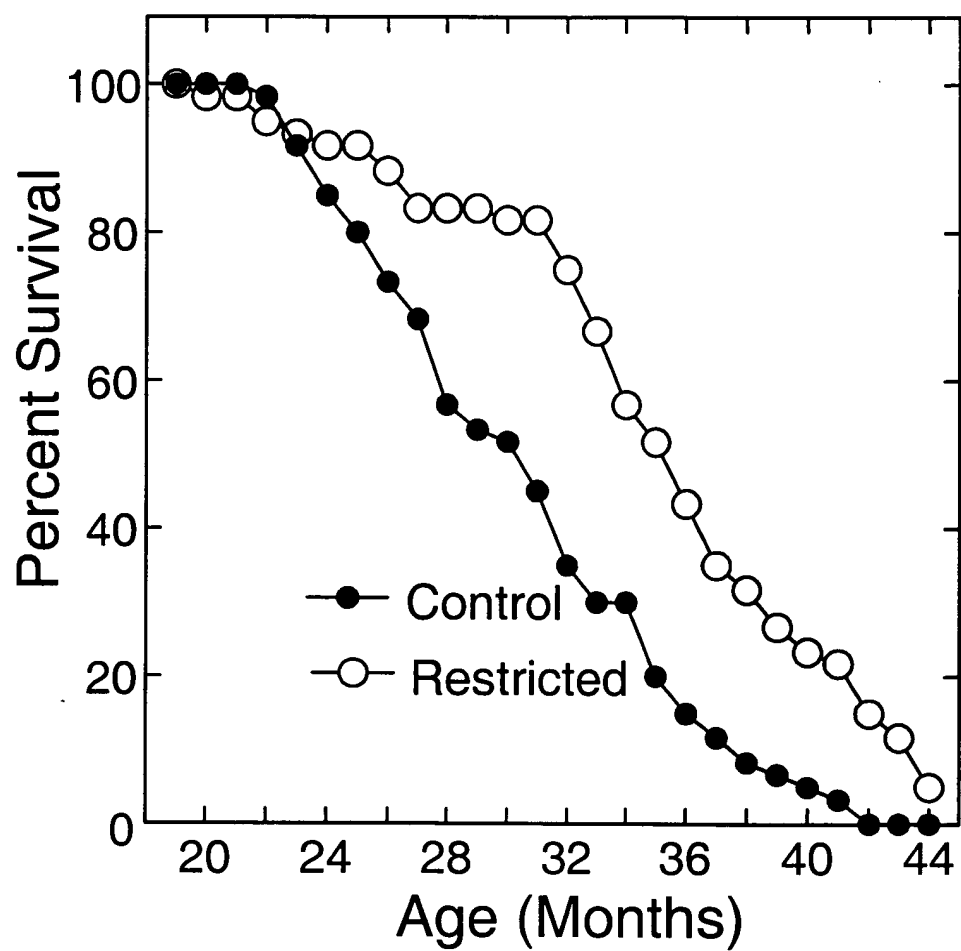


**FIGURE 1**



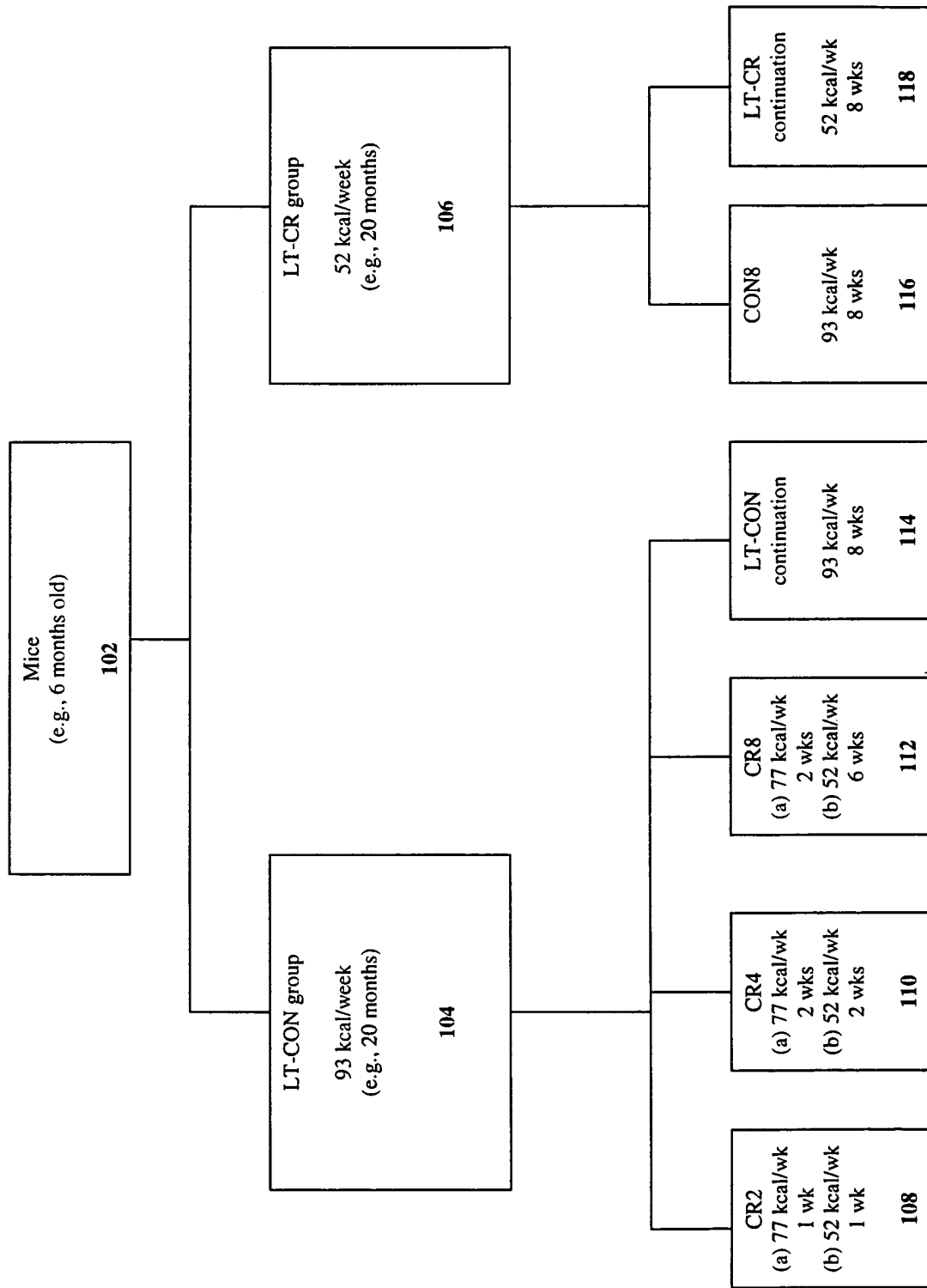


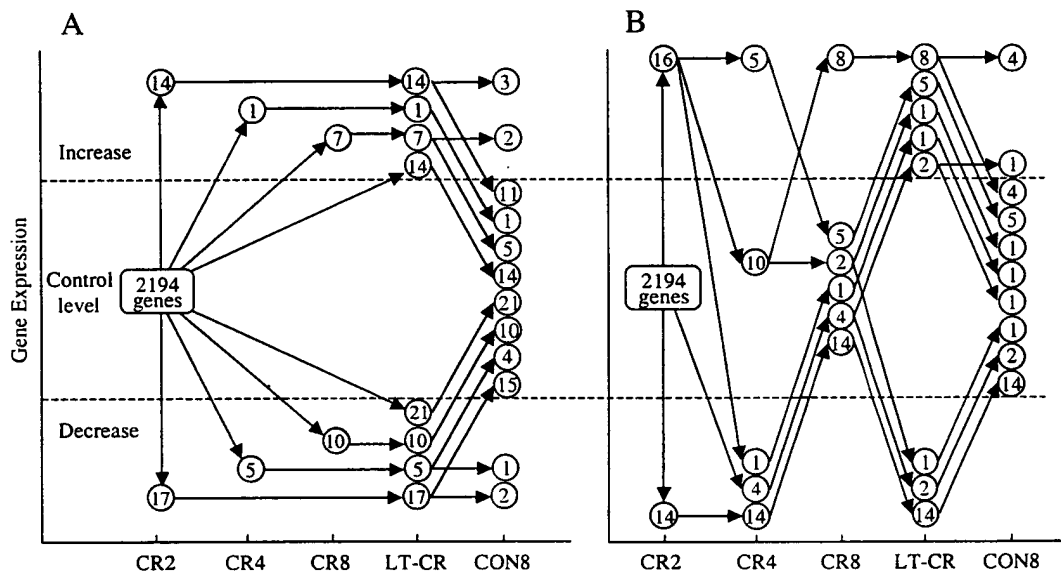
**FIGURE 2** Longevity of mice subjected to caloric restriction from 19 months of age. The CR group is represented by the open circles, while the control group by the filled circles.

100



**FIGURE 3**

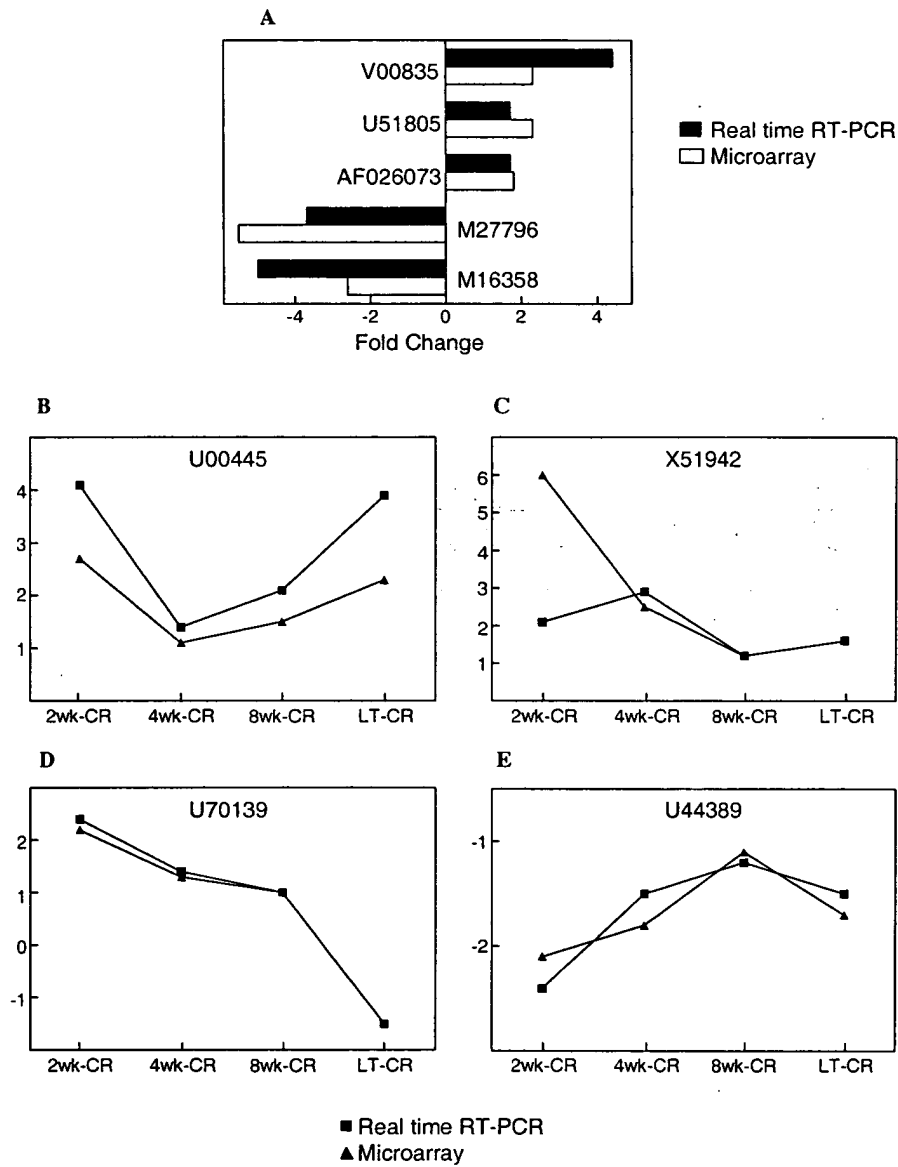




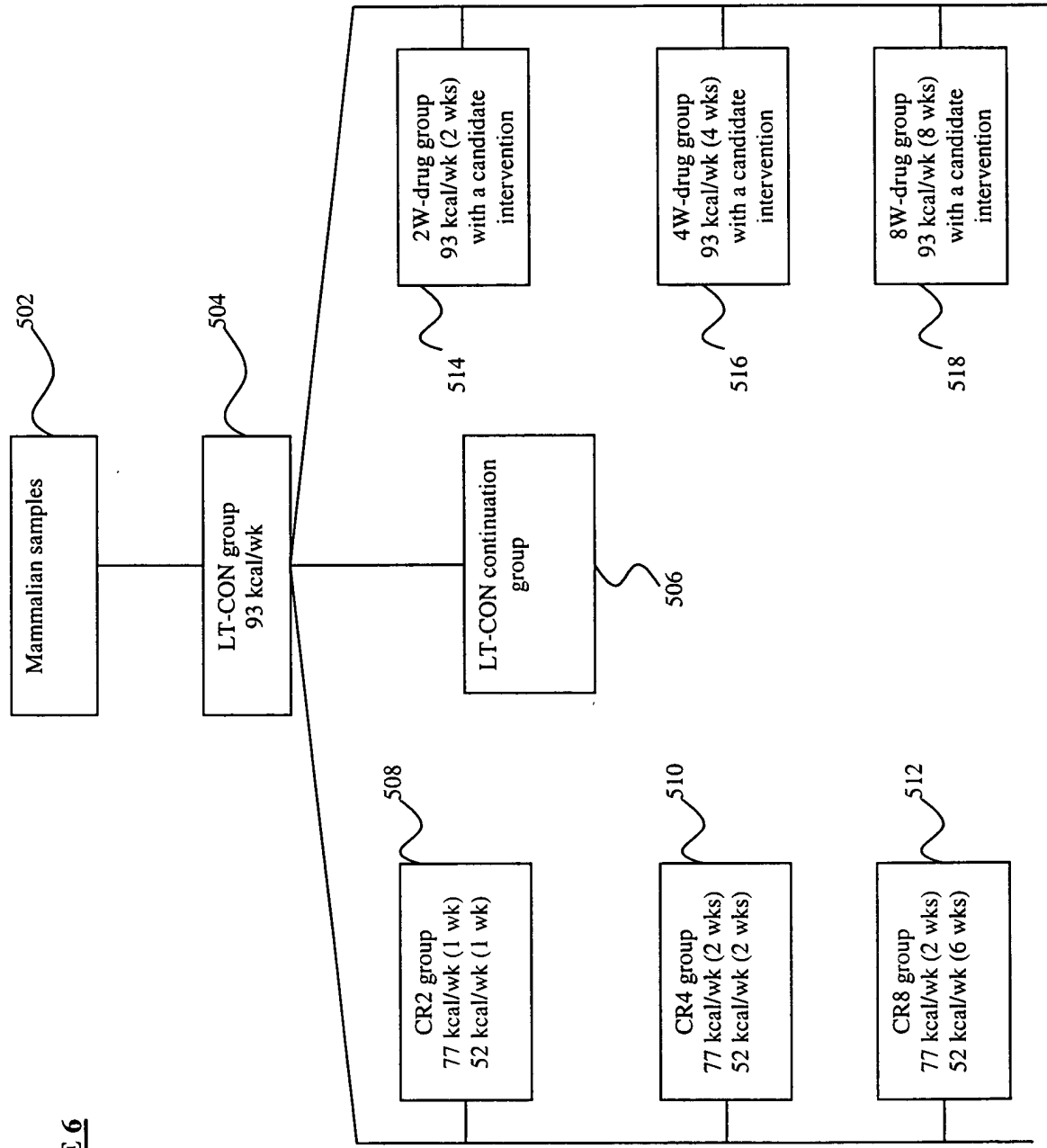
**FIGURES 4A-4B** Dynamics of the early changes in hepatic expression of genes whose expression is affected by LT-CR.

- A) For 54 Affymetrix unique identifiers, early changes in expression initiated after 2, 4 or 8 weeks are sustained through the subsequent time points. For 35 Affymetrix unique identifiers, changes in expression require more than 8 weeks of CR treatment (LT-CR).
- B) For the remaining 34 Affymetrix unique identifiers, there is no consistent pattern after changes in gene expression are initiated after 2, 4 or 8 weeks. The changes in gene expression are not maintained in the same direction through the subsequent time points. An 8-week switch of LT-CR to the control diet segregated the 123 Affymetrix unique identifiers into more clusters (CON8).

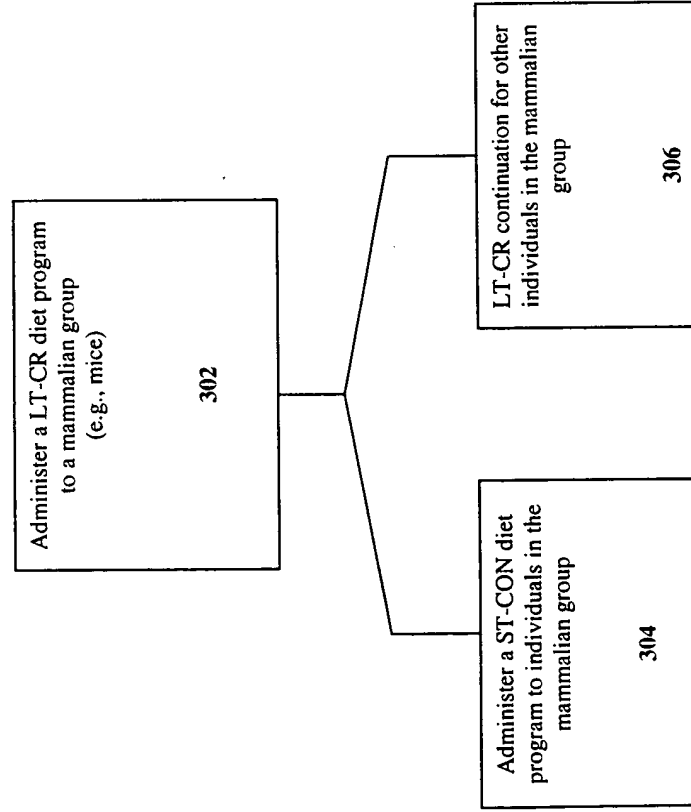
**FIGURES 5A-5E**



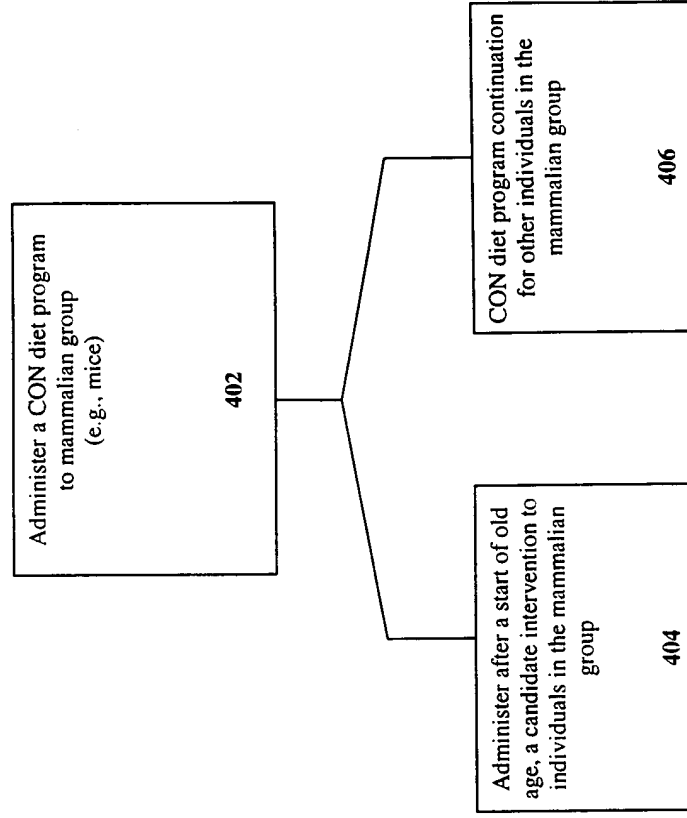
**FIGURE 6**



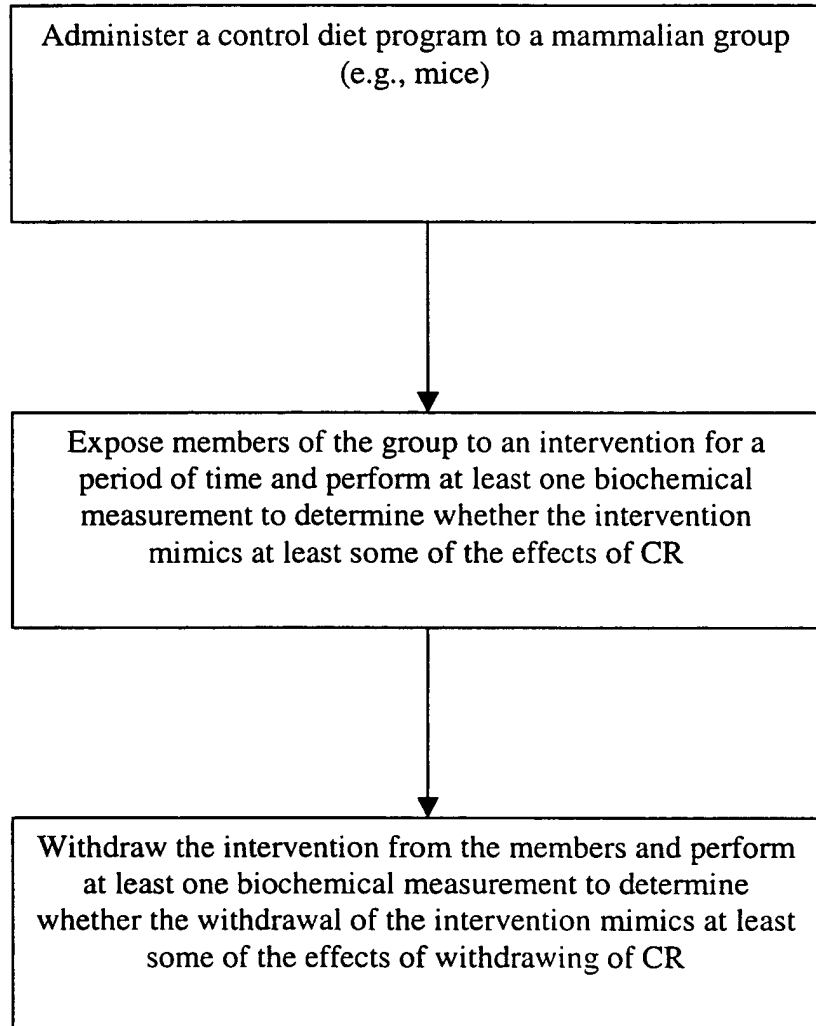
**FIGURE 8**



**FIGURE 7**



**FIGURE 9**





**TABLE 1** Control and CR diet composition

<b>Ingredient</b>	<b>Control*</b>	<b>CR†</b>
Casein	14.0	23.3
L-Cysteine	0.2	0.3
Corn starch	46.6	29.5
Dextrinized cornstarch	15.5	15.5
Sucrose	10.0	10.0
Corn oil (Mazola)	4.0	6.7
Cellulose	5.0	6.8
Mineral mix, AIN-76	3.5	5.8
Choline bitartrate	0.3	0.4
Vitamin mix	1.0	1.7

*Notes:* Values are g ingredient/100 g of diet for these formulations.

\* Mice on the control mice were fed 93 kcal per week of the control diet (AIN-93 M).

†Mice on the CR diets were fed 77 kcal per week of the CR diet or 52 kcal per week of the CR diet (40 % calorie restricted AIN-93M).

**TABLE 2** Primer sequences for qPCR.

Gene Name	GenBank Accession No.	Primer sequences (5'-3') (Forward and Reverse Primers)	PCR Product size (bp)
Metallothionein 1	V00835	CTCCTGCGCCTGCAAGAACTG ACACAGCCCTGGGCACATTTG	96
Arginase 1, liver	U51805	AAAGGAAAGTTCCCAGATGTACCAGG TATAGTGTTCCTCCAGGGTCTACGTCTC	109
Amine N-sulfotransferase	AF026073	CTCTTGCTCTTGAAATGTACAGATATCAGG GGCCACTGAGACTATTCAGACAGG	192
Carbonic anhydrase 3	M27796	CCTTCAAGTAAGGCTCTGAGCTTGC GTGAAATTCATGCTTCTGGGTGAGA	183
Major urinary protein 4	M16358	TTGACTTAACCAAAACCAATCGCTG TGTGAGACAGGATGGAAAGCAGATC	165
Glucose-6-phosphatase, catalytic	U00445	TGTGCTTGCATTCTGTATGGTAGTG AACAGTTGCCTACCAGACACAGCAG	161
Phenylalanine hydroxylase	X51942	GTCTGTTCATTTTTACCTCTCAGGTAAGC AGTTCTCAGAGCCATAATGAATGAATGTAG	156
Carbon catabolite repression 4 homolog (S. cerevisiae)	U70139	TTGCTGATCGAACAGGATGTACACTA AGAAGTCAAAGGCATAGCAAACAGG	111
Transcription elongation factor A, 2	U44389	TCTCCATTACTCGTAAAAGCTCCATAACC GATACAGGAAGGTAGGTTTCATCGTATGG	143
	M18209	CCAGCTGAAATGTAGGCTGTAGCAA ACAGGAGTCTGAACACAGGCAGAAG	199

**TABLE 3 Genes which required more than 8 weeks of CR to change expression**

Category/Gene	Genbank	CR2*	CR4	CR8	CR	CON8
<b>ECM/Cytoskeleton</b>						
Clathrin, heavy polypeptide (Hc)	AA139495	NC	NC	NC	-1.5	NC
<b>Metabolism</b>						
ATP synthase gamma chain, mitochondrial	AA114811	NC	NC	NC	1.5	NC
Cathepsin L	AA096813	NC	NC	NC	2.2	NC
Histidine ammonia lyase	L07645	NC	NC	NC	1.8	NC
Homogentisate 1, 2-dioxygenase	U58988	NC	NC	NC	1.5	NC
Amylase 1, salivary (also called liver alpha-amylase)	V00719	NC	NC	NC	1.5	NC
Tryptophan 2,3-dioxygenase	U24493	NC	NC	NC	1.5	NC
Sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase)	W36875	NC	NC	NC	1.5	NC
Aldehyde dehydrogenase family 1, subfamily A1	M74570	NC	NC	NC	-1.8	NC
Alpha-1-antiproteinase precursor (alpha-1-antitrypsin)	W34969	NC	NC	NC	-1.5	NC
Hydroxysteroid 17-beta dehydrogenase 2	X95685	NC	NC	NC	-1.5	NC
T-complex protein 1, related sequence 1 or acetyl-Coenzyme A C-acetyltransferase 2 (Provisional)	W81884	NC	NC	NC	-1.5	NC
<b>Signal Transducers, Growth Factors</b>						
mVL30-1 retroelement	C77421	NC	NC	NC	1.6	NC
Annexin A7	C78610	NC	NC	NC	-1.5	NC
Ectonucleotide pyrophosphatase/phosphodiesterase 2	AA059550	NC	NC	NC	-1.6	NC
Inhibitor of DNA binding 2	M69293	NC	NC	NC	-1.5	NC
Lymphocyte antigen 6 complex, locus E	U04268	NC	NC	NC	-1.7	NC
Major urinary protein 1	M16355	NC	NC	NC	-2.6	NC
<b>Immune Response, Inflammation</b>						
Complement component 4 binding protein	M17122	NC	NC	NC	-1.8	NC
<b>Stress Response, Xenobiotic Metabolism</b>						
Hydroxyacyl-Coenzyme A dehydrogenase, type II	U96116	NC	NC	NC	2.0	NC
Peroxiredoxin 3	M28723	NC	NC	NC	1.5	NC
Cytochrome P450, 1a2, aromatic compound inducible	X00479	NC	NC	NC	-2.2	NC
Cytochrome P450, 2f2	M77497	NC	NC	NC	-1.8	NC

TABLE 3 continued

Glutathione S-transferase, pi 2	D30687	NC	NC	NC	-1.7	NC
Proteasome (prosome, macropain) subunit, alpha type 2	X70303	NC	NC	NC	-1.5	NC
Proteasome (prosome, macropain) subunit, alpha type 4	AA008321	NC	NC	NC	-1.6	NC
<b>Miscellaneous</b>						
Hemopexin	U89889	NC	NC	NC	1.5	NC
L1 repeat, Tf subfamily, member 14	D84391	NC	NC	NC	1.6	NC
T10	X74504	NC	NC	NC	1.5	NC
EST	AA238331	NC	NC	NC	-1.9	NC
EST	AA213083	NC	NC	NC	-1.7	NC
EST	AA690887	NC	NC	NC	-1.6	NC
EST	AA048018	NC	NC	NC	-1.6	NC
EST	C76068	NC	NC	NC	1.6	NC
EST	C77864	NC	NC	NC	-1.8	NC

\* The average fold change in the specific mRNA derived from all possible pairwise comparisons between the level of gene expression in each dietary group divided by the level of expression in LT-CON. NC is no change.

**TABLE 4** Changes in specific gene expression in stables, genes which responded early to CR and sustained their initial CR-induced expression levels at all subsequent time points.

Category/Gene	Genbank	CR2*	CR4	CR8	CR	CON8
<b>Metabolism</b>						
Apolipoprotein B-100 precursor	AA120586	2.9	3.5	1.5	1.7	1.8
Arginase 1, liver	U51805	3.0	2.3	1.5	2.3	NC
Argininosuccinate lyase	AA237297	2.3	1.5	1.7	2.6	NC
Solute carrier family 10 (sodium/bile acid cotransporter family), member 1	AA117646	1.5	1.5	1.8	1.6	NC
N-sulfotransferase	AF026073	5.0	3.4	1.6	1.8	NC
Fatty acid binding protein 1, liver	AA591003	-4.3	-3.7	-1.7	-1.9	NC
Fatty acid binding protein 1, liver (same gene)	AA087320	-6.8	-6.8	-1.7	-1.8	NC
Carbonic anhydrase 3	M27796	-8.2	-7.5	-1.8	-5.5	NC
2,4-dienoyl-CoA reductase, mitochondrial	AA521793	-2.4	-1.9	-1.6	-1.5	NC
Acetyl-Coenzyme A acetyltransferase 1 (acetoacetyl Coenzyme A thiolase)	AA710204	-2.0	-2.5	-1.5	-1.5	NC
Fatty acid Coenzyme A ligase, long chain 2	U15977	NC	-3.4	-2.0	-1.7	NC
Transglutaminase 2, C polypeptide	M55154	NC	-2.4	-1.5	-1.5	NC
Argininosuccinate synthetase 1	M31690	NC	NC	2.0	2.3	NC
Argininosuccinate synthetase 1 (same gene)	M31690	NC	NC	2.1	2.7	1.5
Carbonyl reductase 1	U31966	NC	NC	1.7	1.7	NC
Glycine N-methyltransferase	W14826	NC	NC	1.8	2.0	NC
S-adenosylhomocysteine hydrolase	L32836	NC	NC	1.5	1.9	NC
<b>Signal Transducers, Growth Factors</b>						
Tumor differentially expressed 1	L29441	2.4	1.8	1.5	1.8	NC
Phosphatase and tensin homolog	U92437	2.5	1.9	1.5	1.6	NC
Poly A binding protein, cytoplasmic 1	AA106783	3.8	2.4	1.6	1.7	NC
Major urinary protein 4	M16358	-3.5	-2.5	-1.6	-2.6	NC
Major urinary protein 4 (same gene)	M16358	-4.0	-2.5	-1.5	-2.7	NC
Thyroid hormone receptor alpha	W13191	-3.6	-2.1	-1.5	-2.1	-1.6
Transthyretin	D89076	-2.3	-2.2	-1.5	-1.8	NC
Major urinary protein 5	M16360	-2.4	-1.5	-1.7	-2.2	NC
MORF-related gene X	AA529583	NC	NC	-1.6	-1.7	NC
Eph receptor B4	Z49085	NC	NC	-1.5	-1.5	NC
X-box binding protein 1	AF027963	NC	NC	-1.5	-1.8	NC
X-box binding protein 1 (same gene)	AA016424	NC	NC	-1.5	-1.6	NC
<b>Immune Response, Inflammation</b>						
Orosomucoid 1	M27008	3.3	1.5	2.0	2.0	1.5

TABLE 4 continued

Complement component 9	X05475	NC	NC	-1.5	-2.0	NC
Interferon-inducible GTPase	AA415898	NC	NC	-1.5	-1.9	NC
Isocitrate dehydrogenase 2 (NADP+), mitochondrial	U51167	NC	NC	1.5	1.5	NC
<b>Stress Response, Xenobiotic Metabolism</b>						
Cytochrome P450, 2b13, phenobarbital inducible, type c	M60358	1.6	1.8	1.8	2.0	NC
P450 (cytochrome) oxidoreductase	D17571	2.8	1.7	1.5	2.1	1.6
Cytochrome C oxidase subunit VIIb	AA521794	4.3	3.4	1.5	1.6	NC
Metallothionein 1	V00835	4.3	2.9	1.6	2.3	NC
Glutathione S-transferase, mu 2	J04696	1.6	1.8	2.4	1.9	NC
Tumor rejection antigen gp 96 (94 KD glucose-regulated protein)	W55140	-3.1	-2.6	-1.7	-1.5	NC
Cytochrome P450, 2b9, phenobarbital inducible, type a	M21855	NC	1.6	2.5	2.3	NC
Glucose regulated protein, 58kDa	M73329	NC	-3.0	-1.7	-1.7	NC
Heat shock 70kD protein 5 (glucose-regulated protein, 78kD)	D78645	NC	NC	-1.9	-2.1	-1.5
Heat shock 70kD protein 5 (glucose-regulated protein, 78kD)	D78645	NC	-2.5	-1.9	-2.0	-1.5
DnaJ (Hsp40) homolog, subfamily B, member 11	AA204094	NC	NC	-1.5	-1.5	NC
Calreticulin	X56603	NC	NC	-1.7	-1.7	NC
Calnexin	AA163552	NC	NC	-1.6	-1.7	NC
<b>Miscellaneous</b>						
Arginine-rich, mutated in early stage tumors	AA408789	-3.0	-1.8	-1.9	-2.2	-1.7
ATP-dependent protease LA2	AA120387	-2.1	-1.5	-1.6	-1.7	NC
EST	AA217076	-4.4	-3.2	-1.7	-1.5	NC
EST	AA537958	-1.9	-1.9	-1.5	-1.6	NC
EST	AA711625	-2.1	-1.8	-1.6	-2.2	NC
EST	AA120109	NC	-2.8	-1.6	-2.2	NC
EST	C76068	NC	NC	1.7	2.1	1.5

\* The average fold change in the specific mRNA derived from all possible pairwise comparisons between the level of gene expression in each dietary group divided by the level of expression in LT-CON. NC is no change.

**TABLE 5** Changes in specific gene expression in oscillators, genes which responded early to CR then returned to control levels briefly, before assuming their LT-CR expression levels

Category/Gene	Genbank	CR2*	CR4	CR8	CR	CON8
<b>Metabolism</b>						
Aminolevulinate, delta-, dehydratase	X13752	-2.7	-2.3	NC	-1.6	NC
Cathepsin L	X06086	3.6	1.6	NC	2.3	NC
Cathepsin L (same gene)	X06086	2.3	NC	NC	2.3	NC
Glucose-6-phosphatase, catalytic	U00445	2.7	NC	1.5	2.3	1.8
Hydroxyprostaglandin dehydrogenase 15 (NAD)	U44389	-2.1	-1.8	NC	-1.7	NC
Hydroxysteroid 17-beta dehydrogenase 5	D45850	-1.6	-1.7	NC	-1.8	NC
Lipase, hepatic	X58426	-2.8	-2.4	NC	-1.5	NC
Ornithine aminotransferase	X64837	1.6	NC	1.5	2.0	1.5
Phenylalanine hydroxylase	X51942	6.0	2.5	NC	1.6	NC
Phosphoenolpyruvate carboxykinase 1, cytosolic	AA110781	1.6	-2.3	NC	1.5	NC
S-adenosylhomocysteine hydrolase	AA237376	2.1	2.0	NC	1.6	NC
S-adenosylhomocysteine hydrolase	L32836	1.5	NC	1.5	2.0	NC
Stearoyl-Coenzyme A desaturase 1	M21285	3.3	NC	6.9	7.9	4.2
Stearoyl-Coenzyme A desaturase 1	AA137436	2.1	NC	6.0	6.6	3.8
Thioether S-methyltransferase	M88694	-2.6	-2.8	NC	-1.6	NC
<b>Signal Transducers, Growth Factors</b>						
Serine (or cysteine) proteinase inhibitor, clade A, member 6 (corticosteroid binding globulin precursor))	X70533	-2.9	-6.4	NC	-2.2	NC
Gap junction membrane channel protein beta 2	M81445	-2.6	-2.9	NC	-1.6	NC
Ras homolog gene family, member U	AA240968	-1.9	-2.4	NC	-1.6	NC
Tumor differentially expressed 1	L29441	4.2	NC	1.5	1.9	NC
<b>Immune Response, Inflammation</b>						
Carbon catabolite repression 4 homolog (S. cerevisiae)	U70139	2.2	NC	NC	-1.5	NC
FK506 binding protein 5 (51 kDa)	U36220	2.3	1.6	NC	1.5	NC
Mannose-binding protein A	U09010	-2.3	-1.8	NC	-1.7	NC
<b>Stress Response, Xenobiotic Metabolism</b>						
Aldo-keto reductase family 1, member 13	AA592828	NC	-1.8	NC	-1.5	NC
Cytochrome P450, 2j5	U62294	-1.5	-1.8	NC	-1.7	NC
Cytochrome P450, 3a16	D26137	NC	-1.9	NC	1.7	NC
Cytochrome P450, 7b1	U36993	-1.5	-1.9	NC	-2.3	NC
Cytochrome P450, steroid inducible 3a11	X60452	NC	-3.2	NC	1.9	1.5
Cytochrome P450, steroid inducible 3a13	X63023	4.1	NC	1.6	2.1	NC

TABLE 5 continued

Esterase 31	L11333	-2.4	-2.2	NC	-2.2	NC
Flavin containing monooxygenase 5	U90535	2.1	NC	1.5	1.5	NC
Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	AA254963	NC	-1.8	NC	-1.6	NC
Solute carrier family 22 (organic cation transporter), member 1	U38652	-2.6	-2.2	NC	-1.6	NC
<b>Miscellaneous</b>						
EST	AA097626	5.8	2.0	NC	1.7	NC
Hepcidin antimicrobial peptide	W12913	-1.5	-1.7	NC	-2.4	NC

\* The average fold change in the specific mRNA derived from all possible pairwise comparisons between the level of gene expression in each dietary group divided by the level of expression in LT-CON. NC is no change.